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Short Communication

# Intermediate and paratenic hosts in the life cycle of *Aelurostrongylus abstrusus* in natural environment

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ABSTRACT

The cat lungworm *Aelurostrongylus abstrusus* affects the domestic cats and other felids all over the world. Feline aelurostrongylosis is of importance in clinical feline medicine. Snails and slugs are the intermediate hosts, but the cat is probably infected by eating paratenic hosts, e.g., rodents, birds, amphibians and reptiles. Herein we present the first finding of *A. abstrusus* in a naturally infected invasive synantropic slugs *Arion lusitanicus* (intermediate host) and wild living rodents *Apodemus agrarius* (paratenic host). The results confirm the usefulness of molecular approaches for investigating the biology, ecology and epidemiology of *A. abstrusus*, the agent of feline aelurostrongylosis.



## 1. Introduction

*Aelurostrongylus abstrusus* (Railliet, 1898) is a metastrongylide nematode parasite, which is known to infect the lungs of domestic cats and wild felids such as the Amur cat (*Felis bengalensis euptilurus*) (Gonzáles et al., 2007) and the Eurasian lynx (*Lynx lynx*) (Szczęsna et al., 2007). Symptoms in definitive hosts infected by *A. abstrusus* are not always detectable, as the disease may be asymptomatic or subclinical. Respiratory signs, e.g. mild to intense cough, sneezing, mucopurulent nasal discharge, dyspnea, open-mouthed abdominal breathing, and even death, are most often observed in young, debilitated or immunosuppressed animals (Traversa and Guglielmini, 2008; Traversa et al., 2010). The life cycle of *A. abstrusus* is indirect. The oviparous females produce eggs in the respiratory system (terminal bronchioles and alveoli) of the definitive felid host and the first-stage larvae (L1s) hatch within the airways of the lung. L1s then migrate via the bronchial/tracheal escalator to the pharynx, are swallowed and passed in the feces. The intermediate hosts are gastropods (snails or slugs), infected through penetration of the epidermis or ingestion of first stage larvae. After entering the intermediate host, L1s will develop into third-stage larvae (L3s) given favorable environmental conditions (Anderson, 2000; López et al., 2005) and encyst mainly in the intestinal ligament (Thiengo et al., 2008). Experimental infections have revealed that mice, frogs, toads, snakes, lizards, ducklings, chickens can serve as paratenic hosts following the ingestion of infected snails or slugs (Hobmaier and Hobmaier, 1935; Gerichter, 1949; Mackerras, 1957; Anderson, 2000). It seems highly possible that cats become infected by the ingestion of infected mice or birds (Bowman, 2008). In cats, the infective larvae penetrate the mucosae of digestive system and travel via the lymphatic system to the lungs, where they mature into adults. Presently molecular data on *A. abstrusus* include sequences of mitochondrion complete genome (Jabbar et al., 2013), ITS2 rDNA genes (Iorio and Traversa, 2008), 18S and 28S rDNA (Chilton et al., 2006). Based on ITS2 rDNA region nested PCR test, with specificity of 100%, was

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established for diagnosis of this parasite from feces and pharyngeal swabs (Traversa et al., 2008).

The aim of this survey is to report for the first time the presence of *A. abstrusus* infective larvae in invasive synantropic slugs *Arion lusitanicus* (intermediate host) and wild living rodents *Apodemus agrarius* (paratenic host), thus demonstrating the veterinary importance of both species in the transmission of *A. abstrusus* to felids. Morphological and morphometric analyses were supported by molecular methods.

## 2. Materials and methods

Twenty-four specimens of *A. lusitanicus* were collected by hand in the area of Pruszcz Gdański (54°, 15′53″ N; 18°, 39′13″ E), in September and November 2012. Identification of slugs was based on the structure of internal organs (Wiktor, 2004). Examination was carried out using a stereomicroscope immediately after capture. Slugs were euthanized with chloroform until movements stopped, then foot, body cavity and internal organs were examined for the presence of cysts containing nematode larvae by the compression method. The cysts were broken using dissecting needles to release the larvae. All nematodes were collected alive, washed in physiological salt solution, killed by heat in fresh water (60–70 °C) and examined as temporary slides using Olympus DP25 digital camera coupled an Olympus BX50 light microscope. After the microscopic examination, i.e. 1–2 h, most of the specimens were preserved in 70% ethanol by maximum a few days and next were subjected to molecular examination.

During a parasitological survey conducted in southwestern Poland in Sulistrowice (50°, 50′54″ N; 16°, 44′33″ E) 42 individuals rodents *A. agrarius* (striped field mouse) were captured in Sherman live traps in September and October of 2012. After identification of species the trapped rodents were euthanized and anatomized. The animal procedures were approved by the Local Ethics Committee (No. 48/2012). The digestion technique was used to examine helminth larvae occurrence in liver, brain, muscles, heart and lungs of the rodents. Samples were digested in a solution of 1% HCl (37%) and 1% pepsin (1:10,000 N.F.) in tap water for 2 h at 46 °C under constant stirring. The ratio between tissue (g) and fluid (ml) was approximately 1:10 (Taira et al., 2004). Individual larvae were washed in distilled water ($dH_2O$) and then using light microscopy, were classified to species level.

Identification of nematode larvae from slugs and mice was based on morphological and morphometric parameters, such as the body size and the presence of rod-like structures in the anterior extremity as well as a characteristic rounded knob in the tail end (Ash, 1970; Mackerras, 1957; López et al., 2005; Thiengo et al., 2008).

### 2.1. DNA isolation

DNA was extracted from individual larvae using BioTrace DNA Purification Kit (EURx, Poland) or Genoplast Tissue Genomic DNA Extraction Mini Kit (Genoplast, Poland) following the manufacturer's instructions.

**Table 1**
Sequences of primers used.

| Name | Sequence (5′–3′) | Reference |
|---|---|---|
| NC1 | ACGTCTGGTTCAGGGTTGTT3 | Traversa et al. (2008) |
| NC2 | TTAGTTTCTTTTCCTCCGCT3 | Traversa et al. (2008) |
| AabFor | GTAACAACGATATTGGTACTATG | Traversa et al. (2008) |
| AabRev | GAACTCCTTCACGTGCTACTCG3 | Traversa et al. (2008) |
| COINF1 | CTTTGCCGGTTTTAGCTGGTGCAA | Present study |
| COINF3 | GGTTGTGTTGTTTGGGCTCA | Present study |
| COINR1 | CCAAATACAGCCCCCAAACTCAAC | Present study |

### 2.2. Nested PCR test

To confirm the morphological identification of larvae derived from *A. lusitanicus* and *A. agrarius*, nested PCR assay specific for an *A. abstrusus* (233 bp region internal to the ITS2) of ribosomal DNA was used. Thermocycling profile and nested PCR procedure were conducted according to Traversa et al. (2008), in Thermal Cycler (TC-512 Techne) in total volume of 25 μl (20 mM Tris–HCl at pH 8.3, 20 mM KCl, 5 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 100 μM of each dNTP) with Hot Taq DNA Polymerase (Genoplast Biochemicals, Poland).

### 2.3. PCR and sequencing COI 1 gene fragment

For all analyzed DNA samples (four from *A. lusitanicus* and two from *A. agrarius*), mitochondrial cytochrome c oxidase subunit I (COI) gene fragments were sequenced. The partial mitochondrial cytochrome c oxidase subunit 1 gene was amplified using forward primer COINF1, COINF3 and reverse primer COINR1 (Table 1). PCR reactions were performed in a TC-512 Techne Thermal Cycler in total volume of 50 μl (70 mM Tris–HCl at pH 8.6, 16.6 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 100 μM of each dNTP), 0.5 μl of each primer at concentration 20 pM/μl, 0.2 μl of Novazym HiFi polymerase (5 units/μl) and 3 μl of template DNA solution. The thermocycling profile was as follows: 3 min denaturation hold at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at 50 °C, 1 min at 72 °C; and 5 min extension hold at 72 °C. Amplified products from the PCRs were electrophoresed on 1.4% agarose gels (Promega, USA) stained with ethidium bromide (0.5 μg/ml). A 100 bp ladder (Novazym, Poland) was loaded in each gel, then photographed under UV light with a gel documentation system (GL 200, Kodak, USA). The amplified products were purified using a PCR Purification Kit (Genoplast, Poland) according to the manufacturer's instruction.

Sequencing reactions were performed for both strands and sequenced on Applied Biosystems ABI Prism 373xl automated sequencer using ABI BigDye™ (Applied Biosystem) chemistry according to manufacturer's protocols. Sequences were assembled and aligned using Vector NTI Advance 11.0 (Invitrogen, Life Technologies). To elucidate any similarities in sequences obtained from the larvae with other metastrongylid sequences previously published in GenBank, a BLAST search was carried out (Thompson et al., 1994). DNA products were sequenced in both directions using the PCR primers and submitted to GenBank/EMBL (accession numbers: KF316480, KF316481, KF318031).